

First molecular evidence that *Juniperus communis* var. *communis* from the eastern hemisphere is growing in the northeastern United States:

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ABSTRACT

Scattered in floras of New England and West Virginia are reports of upright trees of *Juniperus communis*. The diagnostic DNA sequence, petN-psbM, distinguishes all eastern hemisphere *J. communis* varieties from those in the western hemisphere. Analyses of petN-psbM for several upright *J. communis* trees from New England and West Virginia confirmed that trees from Maine and West Virginia were *J. communis* var. *communis* and were likely seed from trees cultivated in cemeteries and gardens. However, four trees from Massachusetts and one from Vermont proved to be upright forms of *J. communis* var. *depressa* (from the western hemisphere). The historical introduction of *J. c.* var. *communis* is discussed as well as a morphological key to *J. communis* varieties. Published on-line www.phytologia.org *Phytologia* 98(1):8-16 (Jan 5, 2016). ISSN 030319430.

KEY WORDS: *Juniperus communis* var. *communis*, *J. c.* var. *depressa*, introduced species, DNA, sequence, petN-psbM.

The genus *Juniperus* is diverse with approximately 67 species (Adams, 2014). *Juniperus communis* is the only species that grows in both eastern and western hemispheres. It is composed of approximately 10 varieties, with 5 in the eastern and 5 in the western hemisphere (Adams, 2014).

Genetic analysis of Arctic populations of *J. communis* (Adams et al., 2003) revealed that these populations clustered by continent, with the populations in Greenland and Iceland showing the highest affinities to populations from Europe and not to those from North America. Adams et al. (2003) concluded that the post-Pleistocene populations on Greenland and Iceland came from Europe and not North America. Adams and Pandey (2003) analyzed *J. communis* and its varieties by use of RAPDs and found considerable variation, but several of the varieties were not discernable.

the result of collaboration between these authors in supplying materials of *J. communis* from upright trees in Maine, Massachusetts, Vermont and West Virginia.

Rosbach (1963) described *J. communis* near Helvetia, Randolph Co., WV: "these obvious columnar trees, though wild, are presumably escapes from the yards and cemeteries of the local Swiss farmers...". Bartgis and Hutton (1988) reported another population of *J. communis* trees in Pendleton Co. WV as well as Highland Co., VA (T. Wieboldt, pers. comm.) and stated that these populations were native. Recent efforts to relocate the Pendleton Co. population were unsuccessful (BS and JV). Prior to this molecular study, all specimens of *J. communis* from Helvetia and Pendleton Co. at West Virginia University Herbarium (WVA) were annotated as *J. communis* var. *communis* based on leaf characters (JV).

Juniperus communis and varieties from the eastern hemisphere are distinguished from *J. communis* varieties in the western hemisphere by a few mutations in the cp region petN-psbM (Adams et al., 2011). The purpose of this study was to examine the petN-psbM region by DNA sequencing to determine if any of the upright tree *J. communis* trees in New England and West Virginia are in fact *J. c.* var. *communis* from Europe or merely upright sports of *J. c.* var. *depressa* (from the western hemisphere).

MATERIALS AND METHODS

Maine:

Art Gilman 07229, Lab Acc Adams 14506, upright tree, 2-3 m, under power line, small popn. on flood terrace, on N side of Austin Stream, 45° 04' 10.8" N; 69° 52' 59" W, 600 ft, Bingham, Somerset Co., ME, 28 Sep 2007;

Massachusetts:

Adams 8569, 4m shrub/tree, some stems vertical, some horizontal, 1.7 mi E of Cushman, MA, on Flat Hill Rd. 0.25mi N of Jct. Flat Hills Rd. & Shutesbury Rd., then 400ft E on private road. 42° 24' 24.22"N, 72° 29' 36.42"W, 427 ft., Hampshire Co., MA, 7 Aug 1998;

Adams 8570, 4m shrub/tree, some stems vertical, some horizontal, 1.7 mi E of Cushman, MA, on Flat Hill Rd. 0.25mi N of Jct. Flat Hills Rd. & Shutesbury Rd., then 400ft E on private road. 42° 24' 24.22"N, 72° 29' 36.42"W, 427 ft., Hampshire Co., MA, 7 Aug 1998;

Adams 8571, 2.5 m tall x 2 m wide shrub, other typical shrubs of *J. c.* var. *depressa* in the population, 1.7 mi E of Cushman, MA, on Flat Hill Rd. 0.25mi N of Jct. Flat Hills Rd. & Shutesbury Rd., then 400ft E on private road. 42° 24' 24.22"N, 72° 29' 36.42"W, 427 ft., Hampshire Co., MA, 7 Aug 1998;

Hickler ns, Lab Acc. Adams 14549, upright tree, 1.5 m tall, female, on Charlemont Isl. in Deerfield River, 1 mi. east of Charlemont, or 2 mi nw of Shelburne Falls, 42° 37' 13" N; 72° 46' 18" W, 477 ft, Franklin Co., MA, 17 May 2015;

Vermont:

Art Gilman 05002 and E. C. Briggs Lab Acc Adams 14507, single large tree in open pasture, Rice Hill Rd., Hartland, near Vermont, New Hampshire border. 43° 32' 12" N; 72° 24' 52" W, 690 ft, Windsor Co., VT, 29 Apr 2005;

West Virginia:

Brian P. Streets 5462, Lab Acc. Adams 14503, Otter Creek Wilderness Area, Monongahela Nat. For., sedge fen, w *Picea rubens*, *Rubus hispidus*, *Carex*. 38° 58' 53.29" N; 79° 39' 36.22" W, 3250 ft, Randolph Co., WV, 4 Nov 2014;

Brian P. Streets 5463, Lab Acc. Adams 14504, Helvetia, WV, ca. 0.2 air mi. from jct CR 45 and CR46. in CR 46 ditch-line, overhung by oak forest. 38° 42' 15.87" N; 80° 11' 52.56" W, 2240 ft, Randolph Co., WV, 14 Jan 2015;

Brian P. Streets 5543 Lab Acc Adams 14509, Lower Gladys, farm off Sulley Rd (CR 1/2), near cemetery, s of Three Springs Run, in old field. 38° 57' 17.47" N; 79° 37' 07.09" W, 2690 ft, Randolph Co., WV, 25 Feb 2015;

Jim Vanderhorst 7892 Lab Acc Adams 14510, Lower Glady, farm off Sulley Rd (CR 1/2), near cemetery, s of Three Springs Run, in old field. 38° 57' 17.47" N; 79° 37' 07.09" W, 2690 ft, Randolph Co., WV, 25 Feb 2015;

Voucher specimens are deposited at the Baylor University herbarium (BAYLU).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN, trnD-T, trnL-F, trnS-G) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM MgCl₂ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. The primers for trnD-trnT, trnL-trnF and trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010).

The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com). NJ (Neighbor Joining analysis was the MAFFT analysis package (<http://mafft.cbrc.jp/alignment/server/>)).

RESULTS AND DISCUSSION

Sequencing petN-psbM yielded 717 bp. NJ analysis using petN-psbM data shows that the Cushman, MA; Hartland, VT and Deerfield River, MA plants group with *J. communis* from the western hemisphere (boldface, Fig. 2).

However, Bingham, ME; Otter Ck., WV; and Lower Glady, WV group with *J. communis* of the eastern hemisphere (boldface Fig. 2). This supports the thesis that these plants are escaped cultivars of *J. c. var. communis*.

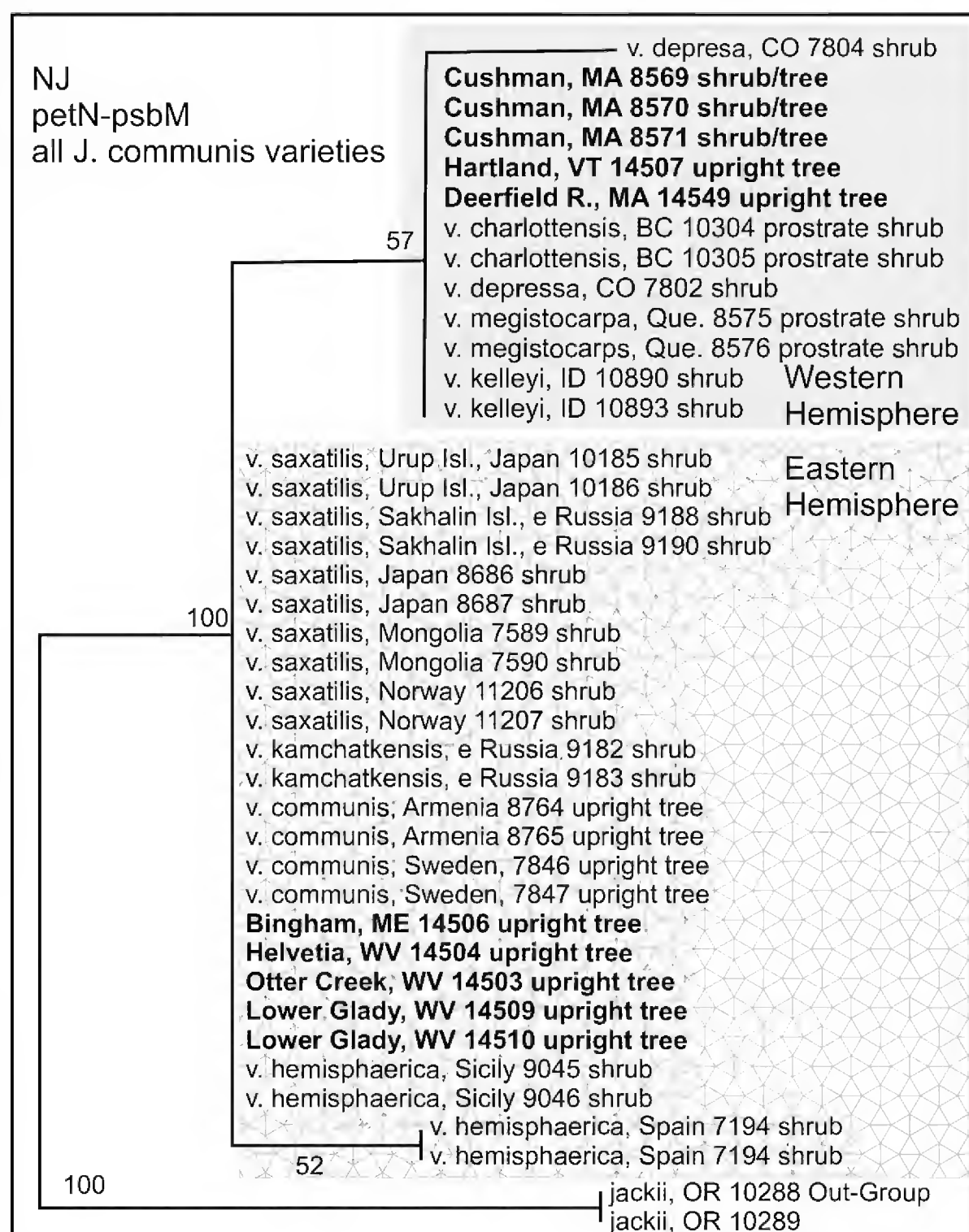


Figure 2. NJ clustering of US *J. communis* trees with all other varieties of *J. communis* using petN-psbM sequence data.

A point mutation is present at position 533 in the petN-psbM sequence. The nucleotide is an A for Cushman, MA, Hartland, VT and Deerfield River, MA and all the *J. communis* varieties in the western hemisphere (Table 1). Position 533 is a T for Helvetia, Otter Creek, and Lower Glady, WV as well as Bingham, ME and all *J. communis* varieties in the eastern hemisphere (Table 1). This provides evidence that these *communis* trees are from seed from cultivated *J. c. var. communis* trees.

Table 1. Nucleotide substitutions and indels in *J. communis* from eastern and western hemispheres. Upright shrubs and trees from northeastern US are in **boldface**.

<u>From Western Hemisphere</u>	<u>position 533</u>	<u>positions 541-545(5 bp indel)</u>
Cushman, MA 8569 upright shrub	A	-----
Cushman, MA 8570 upright shrub	A	-----
Cushman, MA 8571 upright shrub	A	-----
Hartland, VT 14507 upright tree	A	-----
Deerfield R., MA 14549 upright tree	A	-----
<u>All native w hemi. varieties of J. communis:</u>		
var. Charlottensis, BC 10304 prostrate shrub	A	-----
var. Charlottensis, BC 10305 prostrate shrub	A	-----
var. depressa, Colorado 7802 shrub	A	-----
var. megistocarpa 8575 prostrate shrub	A	-----
var. megistocarpa 8576 prostrate shrub	A	-----
var. depressa, Colorado 7804 shrub	A	-----
var. kelleyi, Idaho, 10890 shrub	A	-----
var. kelleyi, Idaho, 10893 shrub	A	-----
<u>From Western Hemisphere but like Eastern Hemisphere <i>J. communis</i> var. <i>communis</i></u>		
Helvetia, WV 14504 upright tree	T	-----
Bingham, ME 14506 upright tree	T	-----
Otter Creek, WV 14503 upright tree	T	CTTCT
Lower Glady, WV 14509 upright tree	T	CTTCT
Lower Glady, WV 14510 upright tree	T	CTTCT
<u>From Eastern Hemisphere, all <i>J. communis</i> varieties:</u>		
var. communis, Sweden, 7846 upright tree	T	-----
var. communis, Sweden, 7847 upright tree	T	-----
var. communis, Armenia 8764, upright tree	T	-----
var. communis, Armenia 8765, upright tree	T	-----
var. hemispherica, Sicily 9045, shrub	T	-----
var. hemispherica, Sicily 9046, shrub	T	-----
var. hemispherica, Spain 7194, shrub	T	-----
var. hemispherica, Spain 7195, shrub	T	-----
var. kamchatkensis, e Russia 9182, shrub	T	-----
var. kamchatkensis, e Russia 9183, shrub	T	-----
var. saxatilis, Urup Isl., Japan 10185, shrub	T	-----
var. saxatilis, Urup Isl., Japan 10186, shrub	T	-----
var. saxatilis, Sakhalin Isl., e Russia 10188, shrub	T	-----
var. saxatilis, Sakhalin Isl., e Russia 10190, shrub	T	-----
var. saxatilis, Japan 8686, shrub	T	-----
var. saxatilis, Japan 8687, shrub	T	-----
var. saxatilis, Mongolia 7589, shrub	T	-----
var. saxatilis, Mongolia 7590, shrub	T	-----
var. saxatilis, Norway 11206, shrub	T	-----
var. saxatilis, Norway 11207, shrub	T	-----

Figures 3-8 show verified *J. c.* var. *communis* from West Virginia and Maine.



Fig. 3. var. *communis*, Otter Creek, WV



Fig. 4. Habitat, var. *communis*, Otter Creek, WV



Fig. 5. v. *communis*, close-up, Otter Creek, WV.



Fig. 6. v. *communis*, close-up, Lower Glady, WV



Fig. 7. var. *communis*, Bingham, ME



Fig. 8. Google Earth 'street view' of power-line near Bingham, ME. Arrow points possible *J. c.* var. *communis* tree.

Juniperus c. var depressa, although normally a shrub, can be a small tree in New England. Figures 9-11 show trees in Hartland, VT (Fig. 9), Cushman, MA (Fig. 10) and Charlemont Island, Deerfield River, MA (Fig. 11).

It is interesting that the three trees from WV all contain an indel (CTTCT) not found in any other varieties (Table 1). It seems likely that these trees arose from seed of a single parent (or group of siblings) that also have the indel. The *J. c. var. communis* trees at Lower Glady and Helvetia, WV appear to have originated from seed dispersed by birds (Adams and Thornburg, 2010) from *var. communis* cultivated at nearby homes and cemeteries (Fig. 12). Columnar trees such as *Cupressus sempervirens*, *Juniperus chinensis* (strict cultivars) and *J. c. var. communis* (strict trees) are popular plantings in cemeteries in the US.



Fig. 9. *var. depressa* tree, Hartland, VT



Fig. 10. *var. depressa*, note 2 upright stems, Cushman, MA



Fig. 11. *var. depressa*, Charlemont Isl., Deerfield River, MA



Fig. 11. Elkins, WV cemetery with cultivated *J. c. var. communis* trees.

The Otter Creek, WV population of *J. c.* var. *communis* consists of hundreds to thousands of trees of various sizes in a remote Wilderness Area. Although it is less than 3 air miles from the Lower Glady site and could have originated from seed transported from this site by birds, it is also possible that it was planted in Otter Creek prior to Wilderness designation during the logging boom around 1900. In any case, the population is robust and expanding in wetland habitat, to the extent that it may displace native vegetation at the site.

As an aid for the identification of *communis* trees, a key to *communis* varieties in North America is presented (including var. *communis*, an escaped, cultivated tree in the ne US).

- 1a. seed cones 10 – 13 mm diam., much larger than leaf length, known only from southeastern Canada.....var. *megistocarpa*
- 1b. seed cones 6 – 9 mm diam., smaller or about equal to leaf length, other than se Canada
 - 2a. glaucous stomatal band about as wide to 1.5 x as wide as each green leaf margin, prostrate or low shrub with ascending branchlet tips (or occasionally a spreading shrub), leaves upturned (to 15 mm), rarely spreading, linear to curved, rarely a small, strict tree (to 2-4 m) in the New England.....var. *depressa*
 - 2b. glaucous stomatal band twice or more as wide as each green leaf margin, upright shrubs or spreading, mat-like shrubs or introduced strict trees (var. *communis*)
 - 3a. glaucous stomatal band twice or more as wide as each green leaf margin, boat-shaped, curved leaves, mature seed cones length greater than leaf length, spreading, mat-like shrub, grows in muskeg bogs, Calvert Island to Queen Charlotte Islands, and north to, Chichagof Island, Alaska.....var. *charlottensis*
 - 3b. glaucous stomatal band 2,3, 4 times as wide as each green leaf margin
 - 4a. strict (columnar) trees, leaves long (15-20 (30) mm), straight (not curved), stomatal band 2 - 3 x as wide as green leaf margin.....(escaped cult. in ne US).....var. *communis*
 - 4b. shrubs in western US, leaves short (<15 mm), curved, stomatal band 3-4 x as wide as each green leaf margin,
 - 5a. gland on brown sheath elongated oval or if a long narrow gland, then with a rounded bottom end, immature seed cones globose, leaves most straight to slightly curved, not usually boat-shaped, free (not appressed to stem or leaf above on branchlet), usu. shrubs to 0.5 m tall with upturned to elevated branchlets, not on serpentine, but grows various habitats from granite, sandstone, alluvial, sand, and lava.....var. *kelleyi* (prev. treated as var. *saxatilis*)
 - 5b. gland on brown sheath long, narrow, raised; immature seed cones elongated-subglobose, leaves curved, boat-shaped, appressed to stem or leaf above on branchlet; shrubs, usually prostrate or mat-like on serpentine or ultramafic rock (sometimes on volcanic lava, rarely on granite.....*J. jackii* (included in this key as it is often confused with var. *kelleyi*)

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